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CONTROLLED MUTATION OF BACTERIA OF THE PASTEURELLA GROUP

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Vegetative hybridization affords the possibility of bringing about the controlled mutation of living forms, including microorganisms.

At present, a large number of facts have been accumulated by Soviet scientists which confirm the proposition that the application of the method of vegetative hybridization in microbiology must be regarded as promising.

Gracheva (2) showed that, during prolonged culturing of *B. coli* communae on a synthetic medium containing killed *B. Breslau* and rabbit serum as the source of nitrogen, *B. coli* acquired the biochemical, virulent, and antigenic characteristics of *B. Breslau* [one of the bacteria of the paratyphoid group.] N. F. Gamaleya (1) considered this result an example of the vegetative hybridization of microorganisms.

Analogous data was also obtained by Timakov (5), Kalina (3) and others, who converted one species of microorganism into another by nurturing the first species on the products of the breakdown or dissociation of the second, particularly on their specific nucleoproteids.

In this article, we present the results of our research on the vegetative hybridization of two microorganisms, *B. coli* and *Pasteurella bovissepticum* which belong to different species far removed from each other. These results were obtained by adding the products of the breakdown of *B. coli* to a culture medium containing *Pasteurella bovissepticum* and vice versa.

To culture the strains being exposed to each other's influence, we used a synthetic Tirode medium. Each test tube was filled with 5 ml of the medium which was then autoclaved at 2 atmospheres for 15 minutes. The pH of the media equaled 7-7.2.

P. bovissepticum can not be cultured in the Tirode liquid. The microorganisms die off in large numbers beginning with the 2d to 3d day after inoculation. *B. coli* can be maintained in this medium a little longer; the bacteria die off after the fifth or sixth passage.

A specific quantity of killed microorganisms, in the one case *B. coli* and in the other *P. bovissepticum*, were added to the medium as a source of nitrogen products before seeding. In another series of test tubes, in addition to these microorganisms, 0.25 ml of sterile noninactivated blood serum from healthy rabbits was added to the Tirode liquid.

The microorganisms being used as a source of nitrogen were washed off the surface of the meat-peptone agar (MPA) with Tirode solution after 24 hours of growth at 37°C, titrated against a standard to a concentration amounting to 2 million microorganisms (per milliliter,) and then heated on a water bath at 75°C for 30 minutes in the case of *B. coli* and at 80°C for 30 minutes in that of *P. bovissepticum*. The sterility of the suspensions of microorganisms was checked by seeding them on ordinary culture media. The *P. bovissepticum* suspension was checked still further by injecting 0.5 ml quantities of it subcutaneously into white mice. The suspensions were considered suitable for use if the seeded culture in medium remained sterile and the mice lived for 5 days.

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Two laboratory strains were used in these experiments. In the case of *P. bovissepticum* we used strain 1337, a strain isolated from the blood of cattle, which has been used in our laboratory for a long time as a vaccine strain and is distinguished by a remarkably well developed antigenicity and high virulence, i.e., 0.1 ml of a one-day bouillon culture of this strain kills a white mouse within 18-24 hours and a 2-year-old bull within 24-36 hours. In smears made from the organs and blood of the dead animals well-defined, bipolarly strained coccobacteria can be observed. This microorganism is a gram-negative nonmotile coccobacterium which grows on a slant agar in the form of fine, colorless, transparent colonies (resembling dew) and in meat-peptone bouillon forms a uniformly distributed delicate turbidity. It only ferments glucose forming an acid and gas, does not liquify gelatin, and does not coagulate milk. In the case of *B. coli*, we used strain 1520 which was isolated from the intestines of a calf and was typical for this species of microorganism in all respects.

The plan of the experiments is shown in Table 1.

The initial seeding of the strains being used in the experiment was carried out with a loop from a one-day-old agar culture. Further reseeding was carried out with four drops added from Pasteur pipettes of approximately identical diameter.

After reseeding, the test tubes were kept in a thermostat for 24 hours at a temperature of 37°C and were then kept at room temperatures for 5 days. On the 7th day after the beginning of the experiment, subsequent reseeding was carried out from each test tube into an identical test tube containing an analogous culture medium. The new test tubes were then treated in the same manner as the initial ones. Simultaneously, one or 2 drops of the contents in each of the experimental test tubes were transferred onto Endos medium in individual Petri dishes with a Pasteur pipette and were spread over the entire surface of the medium with a spatula. The dishes were then kept in a thermostat for 24 hours at a temperature of 37°C, after which the colonies were studied. Colonies which were of interest were then reseeded onto slant agar and other media to study their biochemical properties.

Usually, those colonies were isolated which had exhibited uncharacteristic growth phenomena on Endos medium, i.e., when red colonies appeared from the test tubes where *Pasteurella* were being nurtured on killed *B. coli*, or colorless colonies from the test tubes in which *B. coli* were being raised on killed *P. bovissepticum*.

In all, three series of experiments of the same type were carried out. Each series entailed seven passages. In all three series of experiments identical results were obtained in all passages.

The results of our observations can be summed up as follows: *Pasteurella bovissepticum* raised on killed *B. coli* in the presence of rabbit's blood serum remained viable throughout all seven passages, producing a characteristic growth on MPA and not growing on Endo just as the initial culture did not. During the seventh passage the characteristics of the growth on these media change sharply. Fine, red, lactose-decomposing colonies appeared on the Endo medium. On the MPA medium, the growth was more abundant, approximating the growth of microorganisms of the enteric group, which is not characteristic for the initial culture of *P. bovissepticum*. One-day-old bouillon cultures of these red colonies in 2 milliliter doses caused white mice to die within 3 days, and smears of the organs of the mice revealed an abundance of short, well-staining coccobacteria which resembled *Pasteurella* morphologically, but showed absolutely no evidence of bipolar staining. These red colonies are transformants and differ sharply in their biochemical properties from the original culture. They become completely active, and decompose lactose, glucose,

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mannitol, maltose, etc., causing the formation of acid and gas, but do not decompose sucrose or dulcitol. The original culture had different characteristics: it only decomposed sucrose without the formation of gas, possessed a high degree of virulence, and stained bipolarly in smears made from the organs of infected animals.

These same *P. bovissepticum* raised on killed *B. coli*, but in the absence of rabbit's blood serum, do not change their properties after passages. In a culture medium containing only rabbit's blood serum *P. bovissepticum* do not multiply; the hardier specimens survive three or four passages and then die out. In Tirode liquid without a source of nitrogen, they die within the first few days.

B. coli in our experiments, as they have in many other, exhibited a great tendency to mutate. In the presence of killed *P. bovissepticum* and rabbit's blood serum, isolated colonies which did not decompose lactose appeared beginning with the fifth passage. From the seventh passage on they were almost all colorless. In several of them there was a faintly pink spot in the center. In the test tubes in which there was only rabbit's blood serum and no killed *P. bovissepticum* were present, colorless colonies appeared after the second passage, were maintained until the sixth passage, and then completely stopped growing both on Endos medium and on a slant agar medium. In our experiments, *B. coli* were maintained on Tirode liquid without a source of protein through the fifth passage. They then ceased growing on the control media. The colorless colonies of *B. coli* which did not decompose lactose differed in their biochemical properties from the original culture in that they no longer caused gas formation on colored media.

Summing up the data obtained, we see, on the basis of our experience in raising the above mentioned strains, the specific changes presented in Table 2.

The observations carried out by us show that by changing the type of assimilation and metabolism, controlled mutation is possible not only between closely related species of microorganisms, but also between species of pathogenic microorganisms which are entirely unrelated.(4)

Table 1

Test Tube	Basic Nutrient Medium	Source of Nitrogen		Strains Being Raised	
		Killed Microorganisms 2 million/ml	Rabbit's Blood Serum	Past. bovissepticum 1337	<i>B. coli</i> 1520
1	Tirode liquid 5 ml	<i>B. coli</i>	0.25	Strain 1337	--
2	Tirode liquid 5 ml	<i>B. coli</i>	--	Strain 1337	--
3	Tirode liquid 5 ml	Past. bovissepticum	0.25	--	Strain 1520
4	Tirode liquid 5 ml	Past. bovissepticum	--	--	Strain 1520
Control Test Tubes					
5	Tirode liquid 5 ml	--	--	--	Strain 1520
6	Tirode liquid 5 ml	--	--	Strain 1337	--
7	Tirode liquid 5 ml	--	0.25	--	Strain 1520
8	Tirode liquid 5 ml	--	0.25	Strain 1337	--

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Table 2

Mutation Criteria	Past. bovisepcticum		B. coli	
	Before Experiment	After Experiment	Before Experiment	After Experiment
1. Morphology	Coccobacteria Bipolarity Gram-	Coccobacteria Bipolarity Gram-	Short rod Gram-	Unchanged
2. Biochemical properties on media with:				
Glucose	A + G -	A + G +	A + G +	A + G -
Lactose	A - G -	A + G +	A + G +	A - G -
Other sugars*	A - G -	A + G +	A + G +	A + G -
3. Virulence	Kills white mice within 18-24 hours	Kills white mice within 72 hours	--	--

*The medium used contained mannite, maltose, arabinose, xylose and other sugars.

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